

Acute and Chronic Hepatotoxicity Study of Orally Administered Ethanol Extract of *Carica papaya* Seeds in Adult Wistar Rats

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Abstract: *Carica papaya* seed extracts have shown great promise in the quest for the development of natural plant based male contraceptive. This study investigates the acute and chronic hepatotoxicity of ethanol extract of *C. papaya* seeds in adult Wistar rats. The OECD up-and-down method was used in the acute oral toxicity test using 5 nulliparous, nonpregnant female wistar rats weighing between 180 and 200 g and observed for 14 days. The chronic hepatotoxicity study involved the use of 15 male wistar rats which were divided into 3 groups of 5 each. Group I which served as control were orally administered distilled water while groups II and III received 100 and 250 mg/kg/day of ethanol extract of *C. papaya* seeds respectively for a period of 90 days. Histological investigation of the liver and serum levels of AST, ALT and ALP were carried out at the end of the administration. Result obtained for the acute oral toxicity showed no mortality or morbidity. There was no significant loss of fur and skin lesions, nose and eyes appeared clear and normal. There was no diarrhea, convulsion, salivation, tremors, lethargy, sleep or coma and animals did not show any sign of aggression or unusual behavior during handling. For the chronic hepatotoxicity study, the histology of the liver, hematological indices and serum levels of AST, ALT and ALP showed no significant difference between the control and experimental groups. The study thus concludes that ethanol extract of *C. papaya* seeds is non-toxic and safe.

Key words: Acute toxicity, chronic toxicity, *C. papaya* seeds, Wistar rats

INTRODUCTION

The quest for the development of a male contraceptive particularly from natural sources has led to the discovery of the antifertility efficacy of the seeds of some species of *Carica papaya*. The locally available and widely distributed *C. papaya* seed have shown great promise in male contraception in animal models (Lohiya *et al.*, 1994; Udoh and Kehinde, 1999; Pathak *et al.*, 2000; Sharma *et al.*, 2001; Verma and Chinoy, 2001; Lohiya *et al.*, 2002; Lohiya *et al.*, 2006).

Toxicological investigations of acute, sub-chronic, chronic toxicity of methanol sub-fraction of the benzene chromatographic fraction of the chloroform extract of *C. papaya* seeds have shown that there were no associated health hazards due to treatment with methanol sub-fraction in Wistar albino rats (Lohiya *et al.*, 2006; Goyal *et al.*, 2009; Goyal *et al.*, 2010). Due to the proven male contraceptive efficacy and the need to broaden the available options, the present investigation seeks to study the acute and hepatotoxicity of ethanol extract of *C. papaya* seeds in adult wistar rats.

MATERIALS AND METHODS

Experimental animals: Twenty apparently healthy, proven fertile male Wistar rats weighing between 180 and 220 g and 5 nulliparous, non-pregnant female Wistar rats weighing between 180 and 200 g were used in the present study. The animals were bred and housed in polypropylene cages in the animal house of the Department of Human Anatomy, Ahmadu Bello University, Zaria - Nigeria. The animals were fed rat pellet diet and layers mesh, exposed to a 12 h light: 12 h dark cycle and water was provided *ad libitum*. Animals were treated humanely, Veterinary care and supervision were provided throughout the period of study.

Extract preparation: Ripe *C. papaya* fruits of Homestead variety were commercially obtained from a local market in Zaria and authenticated at the Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria. The seeds were removed, shade dried and coarsely powdered. The powdered material was Soxhleted with ethanol in the Department of Pharmacognosy and

Table 1: Dose response and body weight changes to oral administration of ethanol extract of *C. papaya* seeds in adult female Wistar rats

Animals	BW before dose (g)	Dose (mg/kg)	Dose response	BW After Dose (g)
1 st	180	175	Nil	186
2 nd	191	550	Nil	196
3 rd	188	2000	Nil	193
4 th	194	2000	Nil	198
5 th	183	2000	Nil	188

Table 2: Mean Serum AL T, AS T, AL P and some Hematological indices of male Wistar rats after 90 days administration of ethanol extract of *C. papaya* seeds

Parameters	90 Days extract administration		
	Control (n = 5)	100 mg/kg (n = 5)	250 mg/kg (n = 5)
RBC ($\times 10^6/\text{mm}^3$)	4.60 \pm 0.41	4.10 \pm 0.32	5.00 \pm 0.51
PCV (%)	39.00 \pm 1.79	38.00 \pm 1.14	39.20 \pm 1.56
Hg (g/100 mL)	12.88 \pm 0.58	12.62 \pm 0.38	13.02 \pm 0.52
WBC ($\times 10^3/\text{mm}^3$)	8.42 \pm 0.30	7.86 \pm 0.51	7.96 \pm 0.44
AS T (U/L)	27.40 \pm 1.78	31.40 \pm 2.27	30.20 \pm 1.88
AL T (U/L)	32.00 \pm 2.57	38.60 \pm 1.54	39.00 \pm 2.12
AL P (U/L)	10.40 \pm 0.92	10.00 \pm 1.02	11.60 \pm 0.86

Drug Development of Ahmadu Bello University, Zaria. The Soxhleted material was concentrated under reduced pressure and the oily residue used in the investigation in the month of January 2010 in Zaria-Nigeria.

Acute oral toxicity test: The up-and-down method as outlined in the OECD, 2001 guideline for testing of chemicals was used for the acute oral toxicity study. Five nulliparous, non-pregnant female Wistar rats weighing between 180-200 g and kept under 12 artificial light hours and 12 h of darkness were used for the study. The animals were kept in separate polypropylene cages for 7 days prior to dosing to allow for acclimatization. The animal were fasted (only food was withheld) for 12 h and weighed prior to dosing. A single dose of the extract (175, 550, 2000, 2000, 2000 mg/kg) was consecutively administered by gavage using intubation cannula to one animal at a time. Food was withheld for another 4 h after dosing and administration of extract was at a 48 h interval. After each dose, the animals were observed once every 30 min during the first 2 h and once every hour during the second 2 h. The animals were subsequently observed once daily for 14 days and the toxicity signs checked out included changes in skin and fur, eyes, mucus membranes, tremors, convulsion, salivation, diarrhea, lethargy, sleep and coma.

Chronic toxicity study: 15 male Wistar rats weighing between 180 and 240 g were divided into 3 groups of 5 each and designated as group I, II, and III. After 14 days period of acclimatization, the animals in groups II and III were orally administered 100 mg and 250 mg/kg/day ethanol extract of *C. papaya* seeds respectively. The animals in group I which served as control were administered distilled water daily. Due care was taken to ensure that the test groups received the complete dose in the same volume. The period of administration lasted 90 days at the end of which the animal were sacrificed under

chloroform anesthesia in an enclosed chamber. Blood samples for serum chemistry and hematological indices was obtained through cardiac puncture and liver were harvested for histopathological investigation.

Statistical analysis: Data were analyzed with the aid of a statistical package (SPSS 13.0). One way analysis of variance (ANOVA) was used to compare the data obtained within groups and Student's t-test was used to compare data obtained between groups. Differences were considered significant with $p < 0.05$.

RESULTS

No mortality or morbidity was observed in any of the animals used throughout the 14-day observation period following the oral administration of the different doses of the ethanol extract of *C. papaya* seeds. There was no significant loss of fur and skin lesions. Nose and eyes appeared clear and normal. There was no diarrhea, convulsion, salivation, tremors, lethargy, sleep or coma which are signs associated with oral toxicity. Animals did not show any sign of aggression or unusual behavior during handling. The LD50 of the extract of *C. papaya* seed was found to be above 2000 mg/kg and no significant increase in body weight was observed as shown in Table 1. No physical toxicity signs were seen among the animals subjected to chronic oral administration of the ethanol extract of *C. papaya* seeds. The serum levels of AST, ALT and ALP as well as RBC, PCV, Hg and WBC counts obtained among the experimental groups are not significantly different from that obtained from the control group ($p < 0.05$) as shown in Table 2. Histopathological examination of the liver sections from both control and extract treated groups showed a normal cytoarchitecture with irregular,

DISCUSSION

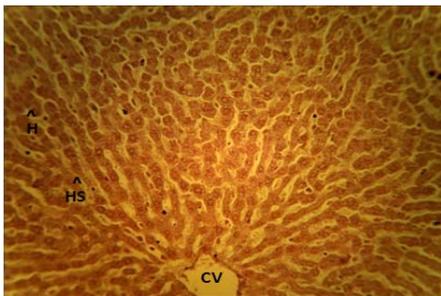


Plate 1a: Transverse section of the Liver of Wistar rats from the control group showing normal Central Vein (CV), Hepatocytes (H) and Hepatic Sinusoids (HS). H & E, X250

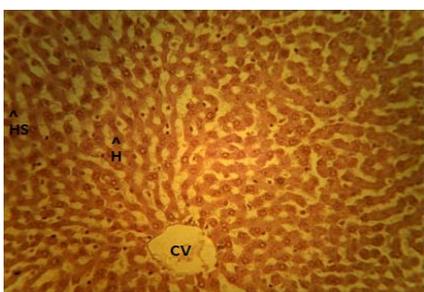


Plate 1b: Transverse section of the Liver of Wistar rats after 90-day oral administration of 100 mg/kg/day *C. papaya* seed extract showing normal Central Vein (CV), Hepatocytes (H) and Hepatic Sinusoids (HS). H & E, X250

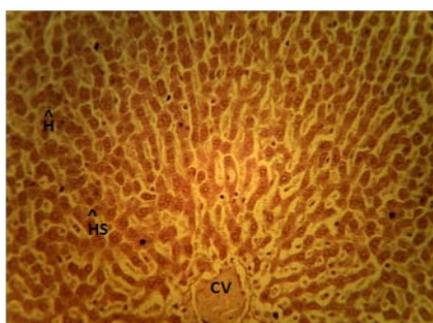


Plate 1c: Transverse section of the Liver of Wistar rats after 90-day oral administration of 250 mg/kg *C. papaya* seed extract showing normal Central Vein (CV), Hepatocytes (H) and Hepatic Sinusoids (HS) H & E, X250

branching, and interconnecting plates of hepatocytes radiating from the central veins. Hepatic sinusoids converging towards the central vein were also observed between the plates of hepatocytes. Present at the portal area were blood vessels surrounded by connective tissues as shown in plates 1a, 1b, and 1c for the control, 100mg and 250 mg/kg/day extract treated groups respectively.

The acute oral toxicity study conducted by administering graded doses of the ethanol extract of *C. papaya* seeds up to 2000 mg/kg body weight over a 14-day observation period did not induce any detectable physical toxicological symptoms like mortality, morbidity, loss of fur, diarrhea, convulsion, salivation, tremor, sleep, coma or aggression. This implies that short term exposure to high doses of the ethanol extract of *C. papaya* seeds does not produce health hazards in Wistar rats. Oral administration of aqueous, methanol, ethyl acetate, chloroform, ethanol have been compared in a toxicity study in rats and only aqueous and chloroform extracts were found to be without significant toxicity (Lohiya *et al.*, 1999, Lohiya *et al.*, 2000). The result in this study is therefore at variance with the reported association between ethanol seed extract of *C. papaya* and toxicity. However, it is pertinent to point out the fact that *C. papaya* possesses specie variation and the reported toxicity associated with the ethanol extract was carried out using the honey dew variety as against the homestead variety used in the present study. The result of the sub-chronic toxicity study which did not show any physical sign of toxicity is a further confirmation of the safety of the ethanol extract of *C. papaya* seeds which is similar to the findings of other workers (Lohiya and Goyal, 1992; Pathak *et al.*, 2000).

The serum levels of Alanine aminotransferase (ALT), Aspartate aminotransferases (AST), and Alkaline Phosphatase (ALP) are usually elevated in conditions associated with injuries or diseases affecting the liver which leads to the release of these hepatocellular enzymes into the bloodstream (Pagana and Pagana, 2002). The normal levels of these enzymes and hematological indices in the treated Wistar rats implies that the ethanol extract of *C. papaya* seed is generally free of anti-metabolic properties which is consistent with the findings of other researchers (Lohiya *et al.*, 2005; Lohiya *et al.*, 2006). This is also consistent with the results obtained from the acute and sub-chronic toxicity studies of the present work.

The hepatocytes are especially liable to injury because of their function of taking up and dealing with many metabolites, drugs and other toxic substances and the result of the present study shows no significant increase in liver weights of the treated groups when compared with the control. Histological analysis of the cellular and extracellular areas of the liver show normal cytoarchitecture in the treated groups as also seen in the control group. The normal histology observed in the liver is in agreement with the normal levels of serum Alanine aminotransferase, Aspartate aminotransferase and Alkaline phosphatase which suggest that ethanol extract of *C. papaya* seeds is not a hepatotoxin.

CONCLUSION

The observed normal histology of the liver, hematological indices, serum levels of AL T, AS T, and ALP as well as the physical examination of Wistar rats exposed to ethanol extract of *C. papaya* seeds inform the decision to conclude in the present study that the extract is not a hepatotoxin and is generally safe.

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